ANTIMICROBIAL EFFECTIVENESS OF KANAMYCIN, AMINOSIDIN, BB-K8, SISOMICIN, GENTAMICIN AND TOBRAMYCIN COMBINED WITH CARBENICILLIN OR CEPHALOTHIN AGAINST GRAM-NEGATIVE RODS

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MANY severe infections in hospital patients, especially those with altered host defences against infection, are caused by Gram-negative bacilli. Bacteria isolated from patients with disseminated tumours, leukaemias, haematosarcomas and other debilitating diseases are often resistant to many commonly used antibiotics. No single drug regimen has yet been found effective enough to be used for the initial treatment of infections thought to be due to Gram-negative bacilli under these conditions; a combination of antibiotics is therefore often given empirically (Schimpff *et al.*, 1971; Tattersall, Spiers and Darrell, 1972).

Another reason for using combinations of antimicrobial agents in the treatment of severe bacterial infections is to take advantage of possible synergistic interaction between them. It has been shown that infections treated with combinations that are synergistic *in vitro* usually have a better clinical outcome than those treated with combinations that are antagonistic or additive (McCabe and Jackson, 1965; Klastersky, Cappel and Daneau, 1972). The investigation of combinations of drugs *in vitro* may be important, therefore, both to assess the antibacterial spectrum of various combinations and to investigate the frequency with which these combinations are synergistic.

The present study explores these two aspects of carbenicillin and cephalothin in combination with different aminoglycosides. Until recently the most commonly used parenteral aminoglycoside antibiotics were kanamycin and gentamicin. Other potentially useful parenteral aminoglycoside antibiotics have now been introduced for investigational purposes. These are tobramycin, sisomicin, BB-K8 and aminosidin. Tobramycin and sisomicin are similar to gentamicin in their antibacterial action and pharmacologically. Comparative studies *in vitro* have shown slight differences in activity especially with respect to *Pseudomonas aeruginosa* (Waitz *et al.*, 1972). BB-K8 and aminosidin are kanamycin-like antibiotics of which the former has much higher activity against this organism.

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MATERIALS AND METHODS

All the bacteria tested in this study were recently isolated from patients at the Institut Jules Bordet, which is the clinical centre for cancer therapy of Brussels University. They include 92 strains of *Escherichia coli*, 91 of *Klebsiella* spp., 93 of *Proteus mirabilis* and 48 of *Ps. aeruginosa*.

The susceptibility of these strains to carbenicillin, cephalothin, kanamycin, aminosidin, BB-K8, gentamicin, sisomicin and tobramycin was determined by the inocula-replicating method (Steers *et al.*, 1959). Mueller-Hinton Agar (BBL) and 10^4 dilutions of overnight cultures were used throughout. The plates were incubated for 18 hours at 37°C. The tests of combined activity were performed by the same technique on the plates containing 2-fold dilutions of the various combinations of the aminoglycoside antibiotics with carbenicillin or cephalothin. The maximum concentrations used were 12 µg per ml for the aminoglycosides, 100 µg per ml for cephalothin, and 400 µg per ml for carbenicillin. These relative concentrations were chosen because they represent multiples of maximum levels of these antibiotics obtainable in the blood during therapy.

A combination was considered *synergistic* when it was at least four times as effective as either drug alone and *antagonistic* when it was four times less effective than either antibiotic alone. In all other instances, the combination was labelled *indifferent*. Representative strains for which synergism was found by the agar dilution technique were further investigated by studies of standard killing curves. Inocula of approximately 10^5 cells per ml of trypticase soy broth were exposed to carbenicillin (25 µg per ml), cephalothin (6 µg per ml) and aminoglycosides (0.7 µg per ml). The same strains were simultaneously tested against various combinations of the aminoglycosides and carbenicillin or cephalothin at the same concentrations of the drugs. At 0, 30, 60, 90, 120, 150, 180, 240 min. and 20 hours after the onset of the study, samples were removed and the number of viable cells ascertained by plate counts.

The same technique was used to investigate the rate at which various aminoglycosides killed representative strains of the four species of bacteria studied.

RESULTS

Antibiotic sensitivities

As indicated in fig. 1, the sensitivities of *E. coli*, and *klebsiella* and *proteus* strains followed a similar pattern. These bacteria were very sensitive to tobramycin, sisomicin and gentamicin. The minimum inhibitory concentration for 90% of these strains (MIC90) was 0.3 or less µg per ml for all three drugs. Tobramycin and sisomicin appeared more active than gentamicin, but the differences were small and therefore of doubtful clinical significance. Against *E. coli* and *klebsiella* strains, BB-K8, kanamycin and aminosidin appeared similarly effective. The median MIC (MIC50) was less than 1 µg per ml but only BB-K8 inhibited all the strains examined at concentrations obtainable in the blood and tissues under clinical conditions. *Proteus* strains showed more conspicuous differences in their sensitivity to kanamycin, BB-K8 and aminosidin but all these antibiotics could be considered effective.

Against *Ps. aeruginosa*, differences in the antimicrobial activity of the six aminoglycosides were more pronounced. Kanamycin and aminosidin had little effect. The MIC50 for tobramycin, sisomicin, gentamicin, and BB-K8 was respectively 0.07, 0.3, 1.5, and 1.5 µg per ml and the MIC90 0.7, 1.5, 8.0 and 4.5 µg per ml respectively.

Bactericidal activity. The bactericidal activity of the six aminoglycoside antibiotics was studied on 12 strains (3 of *E. coli*, 3 of *Klebsiella* spp., 3 of *Pr. mirabilis* and 3 of *Ps. aeruginosa*.) Each strain was exposed to 0.3 and 3.0 μg per ml of tobramycin, sisomicin, and gentamicin and to 1.2 and 12.0 μg per ml of kanamycin, aminosidin and BB-K8 in trypticase soy broth. The lower concentrations were not bactericidal to any of the strains studied. The higher

FIG. 1.—Cumulative percentage of strains of *Escherichia coli* (of 92 examined), *Klebsiella* spp. (of 91 examined), *Proteus mirabilis* (of 93 examined), and *Pseudomonas aeruginosa* (of 48 examined) that were inhibited by increasing concentrations of aminosidin, kanamycin, BB-K8, gentamicin, sisomicin and tobramycin.

concentrations were bactericidal to all strains except that kanamycin and aminosidin were not bactericidal to *Ps. aeruginosa*. No major differences between the rates of killing could be demonstrated, although BB-K8 was the most rapidly bactericidal drug for 10 of the 12 strains studied; within 90 min. of exposure bacteria could no longer be cultivated.

Antibacterial activity of combinations

Spectrum. No important extension of spectrum was detected when carbenicillin and aminoglycosides were combined in proportions (w/w) of 100/12 or 400/12. The percentage of the strains inhibited was similar whether relatively low concentrations (aminoglycoside 0.7 μg per ml; carbenicillin 6 μg per ml;

cephalothin 6 μg per ml) or higher concentrations (aminoglycoside 3 μg per ml; carbenicillin 100 μg per ml; cephalothin 25 μg per ml) were used.

Against *E. coli*, *Klebsiella* spp., and *Pr. mirabilis*, the advantage of the combinations of BB-K8, gentamicin, sisomicin and tobramycin with carbenicillin or cephalothin over the aminoglycosides alone was not dramatic. The aminoglycosides alone or their combinations with carbenicillin or cephalothin inhibited 95% or more of the strains studied. Kanamycin and aminosidin, when tested alone, were active on fewer strains of *E. coli*, *Klebsiella* spp., and *Pr.*

TABLE
Frequency of synergic responses to antibiotic combinations

Antibiotic combination	Percentage of strains on which the stated antibiotic combination acted synergically when tested against			
	92 strains of <i>Escherichia coli</i>	91 strains of <i>Klebsiella</i> spp.	93 strains of <i>Proteus mirabilis</i>	48 strains of <i>Pseudomonas aeruginosa</i>
Carbenicillin plus				
BB-K8	5.4	8.4	15.8	93.6
aminosidin	2.1	1.8	1.8	42.5
kanamycin	25.0	2.8	14.8	44.2
gentamicin	7.6	1.8	11.8	70.2
sisomicin	10.8	4.6	12.8	87.6
tobramycin	13.5	12.1	16.8	95.7
Cephalothin plus				
BB-K8	8.4	31.5	29.0	8.0
aminosidin	8.4	15.0	13.8	0.0
kanamycin	3.7	53.2	14.8	0.0
gentamicin	2.8	53.2	14.8	4.0
sisomicin	16.8	31.5	8.9	4.0
tobramycin	15.8	46.7	16.5	14.8

mirabilis and the addition of carbenicillin or cephalothin to these agents resulted in a marked broadening of antibacterial spectrum. Against *E. coli* and *klebsiella* strains, the addition of cephalothin to kanamycin or aminosidin resulted in a broader antimicrobial activity than the addition of carbenicillin.

As far as *Ps. aeruginosa* is concerned, the activity of aminosidin and kanamycin (which alone had little effect) in combination with carbenicillin probably reflected the activity of carbenicillin itself, although the number of strains inhibited by the combinations was higher than that inhibited by carbenicillin alone. Carbenicillin at concentrations of 6 μg per ml, 25 μg per ml and 100 μg per ml was inhibitory to 6.3, 66 and 80.8% respectively of the strains of *Ps. aeruginosa*. Cephalothin did not increase the action of either aminosidin or kanamycin against pseudomonads.

The advantage of the combinations tobramycin-carbenicillin and sisomicin-carbenicillin over tobramycin or sisomicin alone was difficult to assess because tobramycin and sisomicin were very active alone at relatively low concentrations.

A concentration of 0.7 μg of tobramycin or sisomicin per ml inhibited respectively 93.6 and 82.9% of the strains of *Ps. aeruginosa* tested.

On the other hand, there was a clear advantage in the number of strains inhibited by the combinations of gentamicin-carbenicillin and BB-K8-carbenicillin over gentamicin, BB-K8 or carbenicillin alone.

FIG. 2.

FIG. 3.

FIG. 2.—Killing curves demonstrating synergism between aminoglycosides and cephalothin against a strain of *Klebsiella pneumoniae*: ● --- ● = control; ○ --- ○ = cephalothin 6 μg per ml; Δ — Δ = aminoglycoside 0.7 μg per ml; $\blacktriangle$ — $\blacktriangle$ = cephalothin 0.6 μg per ml + aminoglycoside 0.7 μg per ml.

FIG. 3.—Killing curves demonstrating synergism between BB-K8 and carbenicillin against a strain of *Pseudomonas aeruginosa*: ● --- ● = control; ○ --- ○ = carbenicillin 25 μg per ml; Δ — Δ = BB-K8 0.7 μg per ml; $\blacktriangle$ — $\blacktriangle$ = carbenicillin 25 μg per ml + BB-K8 0.7 μg per ml.

In-vitro synergism. As indicated in the table, synergism *in vitro* occurred most frequently between aminoglycosides and carbenicillin against *Ps. aeruginosa* and between aminoglycosides and cephalothin against klebsiella strains. No antagonism between the antibiotics tested was found. Figs. 2 and 3 show that considerable potentiation of bactericidal action can be demonstrated when organisms are exposed to pairs of antibiotics that exhibit synergism in serial dilution tests.

DISCUSSION

Against the common Gram-negative micro-organisms usually responsible for clinical sepsis (*E. coli*, klebsiella and proteus strains, *Ps. aeruginosa*) aminoglycosides and kanamycin were less active than gentamicin, sisomicin, tobramycin

and BB-K8. Aminoglycosides showed little activity against *Ps. aeruginosa*. It is important to stress that kanamycin, aminoglycosides and BB-K8 can be given without fear of excessive toxicity in higher doses than gentamicin, sisomicin and tobramycin; the respective daily dosages for these two groups of antibiotics are 20 mg per kg and 5 mg per kg. Therefore, it is likely that against many *E. coli*, *Klebsiella* strains and *Pr. mirabilis* all the aminoglycoside antibiotics studied here will be found to be similarly effective. It should be observed, however, that even high concentrations of kanamycin and aminoglycosides were unable to inhibit a substantial proportion of strains of *E. coli* and *Klebsiella* spp.

Against *Ps. aeruginosa*, kanamycin and aminoglycosides were almost without bacteriostatic effect. On a weight-for-weight basis, tobramycin was more active than sisomicin, which was more active than gentamicin and BB-K8, although the last drug, as already mentioned, can be given at higher dosage than the other three. No strain of *Ps. aeruginosa* resistant to BB-K8 has been found, but a few strains were resistant to gentamicin, and some of these were also resistant to sisomicin and to tobramycin. That cross resistance between these drugs exists in some, but not all, strains of *Ps. aeruginosa* has already been reported (Klastersky *et al.*, 1973a).

Sisomicin, tobramycin and BB-K8, at concentrations that can be reached in the blood and presumably in the tissues during therapy, were inhibitory to most of the strains of Gram-negative bacilli tested. However, there is no clear demonstration that under clinical conditions, especially in patients with altered defence-mechanisms, these aminoglycosides would prove as effective against susceptible strains as penicillins, cephalosporins or combinations of aminoglycosides with penicillins or cephalosporins. On the contrary, gentamicin has been shown to be quite ineffective in severe sepsis in granulocytopenic patients (Jackson and Riff, 1971). The reason for this is not completely understood. Presumably, only relatively low antibacterial activities in blood and tissue can be obtained with drugs, such as gentamicin, tobramycin and sisomicin, that are toxic in high dosage. In this respect, the introduction of a drug such as BB-K8, which can be given in higher doses than gentamicin, tobramycin and sisomicin without excessive risk of toxicity, might prove to be important.

The antibacterial spectrum of each of the combinations we tested was always broader than that of the components of the combination. This raises the possibility that in certain circumstances inhibitory and possibly bactericidal action may be obtainable from the combination of two drugs to which the organism is relatively insensitive. This has been described for the action of combinations of penicillins with aminoglycosides on enterococci and is probably best explained by the different sites of action of the two antibiotics (Moellering, Wennersten and Weinberg, 1971).

Synergism between the aminoglycoside antibiotics and carbenicillin or cephalothin in this study was found in about 10–20% of the strains we studied. However, synergism was much more frequent with the aminoglycoside-carbenicillin combinations against *Ps. aeruginosa* and the aminoglycoside-cephalothin combination against *Klebsiella* strains. There were differences in

the frequency with which synergism was found depending on the aminoglycoside drug; BB-K8, sisomicin and tobramycin combined with carbenicillin gave the highest frequency of synergistic action against *Ps. aeruginosa*. A similar influence of the nature of the aminoglycoside on the activity of a penicillin against enterococci has already been reported (Harwick, Kalmanson and Guze, 1973). In enterococcal infections, the clinical significance of in-vitro synergism is well known; this may also be important in infections caused by Gram-negative rods, because synergistic combinations may result in better clinical results than non-synergistic or antagonistic combinations (McCabe and Jackson, 1965; Klastersky *et al.*, 1972).

Our data, however, should not be interpreted as suggesting that any serious infection should be treated with a combination of drugs. Neither should the clinical use of such antibiotic combinations induce in the physician such a state of overconfidence in the therapy that the search for the causative agent of the infectious episode is delayed. Nevertheless, there are well defined situations in which the use of combinations of antibiotics is probably desirable. These may include the empirical treatment of sepsis before the results of bacteriological studies are reported by the laboratory and the treatment of severe infections caused by micro-organisms against which a given combination is presumably synergistic *in vivo*. This is the case with enterococci; the present findings and the results of other recent studies (Klastersky, Swings and Daneau, 1970; Klastersky, Henri and Vandenborre, 1973*b*) indicate that it may also be true of *Ps. aeruginosa* and klebsiella strains.

SUMMARY

The activity of kanamycin, aminosidin, BB-K8, sisomicin, gentamicin and tobramycin, has been compared *in vitro* against a variety of Gram-negative micro-organisms isolated from hospital patients. Clear differences in activity against *Pseudomonas aeruginosa* were observed between these antibiotics, tobramycin, BB-K8 and sisomicin being more active than the others. No major broadening of the spectrum occurred when carbenicillin and cephalothin were combined with the more active aminoglycosides, tobramycin, sisomicin and BB-K8. Synergism was observed against more than 50% of the strains of *Ps. aeruginosa* when BB-K8, tobramycin, sisomicin or gentamicin were combined with carbenicillin and against more than 50% of klebsiella strains when aminosidin, kanamycin or gentamicin were combined with cephalothin.

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